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=> d his

     (FILE 'HOME' ENTERED AT 11:43:12 ON 16 AUG 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:43:37 ON 16 AUG 2005
L1         23701 S THIOREDOXIN
L2         48386 S INCLUSION (A)BOD?
L3           226 S L1 AND L2
L4             2 S "INCLUSION PARTNER PROTEIN"
L5        282010 S INSOLUBLE
L6            55 S L3 AND L5
L7            20 DUP REM L6 (35 DUPLICATES REMOVED)
                 E CHATTERJEE D/AU
L8          1046 S E3
                 E LONGO M/AU
L9           646 S E3
                 E FLYNN E/AU
L10          272 S E3
                 E OBERFELDER R/AU
L11           56 S E3-E7
L12         2013 S L8 OR L9 OR L10 OR L11
L13            4 S L3 AND L12
L14            3 DUP REM L13 (1 DUPLICATE REMOVED)
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| NEWS | 6 | MAR 03 | REGISTRY/ZREGISTRY - Sequence annotations enhanced |
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| NEWS | 21 | JUN 13 | FRFULL enhanced with patent drawing images |
| NEWS | 22 | JUN 27 | MARPAT displays enhanced with expanded G-group definitions and text labels |
| NEWS | 23 | JUL 01 | MEDICONF removed from STN |
| NEWS | 24 | JUL 07 | STN Patent Forums to be held in July 2005 |
| NEWS | 25 | JUL 13 | SCISEARCH reloaded |
| NEWS | 26 | JUL 20 | Powerful new interactive analysis and visualization software, STN AnaVist, now available |
| NEWS | 27 | AUG 11 | Derwent World Patents Index(R) web-based training during August |
| NEWS | 28 | AUG 11 | STN AnaVist workshops to be held in North America |

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FILE 'LIFESCI' ENTERED AT 11:43:37 ON 16 AUG 2005
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=> s thioredoxin
L1       23701 THIOREDOXIN

=> s inclusion (a)bod?
L2       48386 INCLUSION (A) BOD?

=> s l1 and l2
L3         226 L1 AND L2

=> s "inclusionm partner protein"
<------User Break------>

SEARCH ENDED BY USER
SEARCH ENDED BY USER
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=> s "inclusion partner protein"
L4 2 "INCLUSION PARTNER PROTEIN"

=> d 1-2 ibib ab

L4 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:219988 BIOSIS
DOCUMENT NUMBER: PREV200400223055
TITLE: Methods for production of proteins.
AUTHOR(S): Chatterjee, Deb [Inventor, Reprint Author]; Longo, Mary [Inventor]; Flynn, Elizabeth [Inventor]; Oberfelder, Robert [Inventor]
CORPORATE SOURCE: North Potomac, MD, USA
ASSIGNEE: Invitrogen Corporation, Frankfurt am Main, DE, USA
PATENT INFORMATION: US 6703484 20040309
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar 9 2004) Vol. 1280, No. 2. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Apr 2004
Last Updated on STN: 21 Apr 2004

AB The current invention provides methods for producing a polypeptide as inclusion bodies in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an **inclusion partner protein**, such as E. coli thioredoxin or a modified E. coli thioredoxin, such that host cells comprising the gene construct produce the polypeptide as intracellular inclusion bodies. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The invention further provides methods for producing protein molecular weight ladders for use in protein gel electrophoresis, as well as proteins and protein molecular weight ladders produced by these methods.

L4 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:493673 HCAPLUS
DOCUMENT NUMBER: 129:118770
TITLE: Methods for production of recombinant proteins as inclusion bodies in bacterial host cells
INVENTOR(S): Chatterjee, Deb; Longo, Mary; Flynn, Elizabeth; Oberfelder, Robert
PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9830684 | A1 | 19980716 | WO 1998-US492 | 19980108 |

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,

DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG,
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI,
FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM,
GA, GN, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| AU 9860209 | A1 | 19980803 | AU 1998-60209 | 19980108 |
| EP 963435 | A1 | 19991215 | EP 1998-903438 | 19980108 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| JP 2001512306 | T2 | 20010821 | JP 1998-531177 | 19980108 |
| US 2002065392 | A1 | 20020530 | US 1998-4068 | 19980108 |
| US 6703484 | B2 | 20040309 | | |
| US 2004204563 | A1 | 20041014 | US 2003-728766 | 20031208 |
| PRIORITY APPLN. INFO.: | | | US 1997-34658P | P 19970108 |
| | | | US 1998-4068 | A1 19980108 |
| | | | WO 1998-US492 | W 19980108 |

AB The current invention provides methods for producing a polypeptide as inclusion bodies in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an **inclusion partner protein**, such as Escherichia coli thioredoxin or a modified E. coli thioredoxin, such that host cells comprising the gene construct produce the polypeptide as intracellular inclusion bodies. Addnl. **inclusion partner proteins** include gene 32 protein of bacteriophage T4, KpnI methylase, and E. coli Dead-Box protein. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The desired protein can be released from inclusion bodies by chemical cleavage (CNBr or hydroxylamine) or enzymic cleavage (factor Xa, thrombin, enterokinase). The invention further provides methods for producing protein mol. weight ladders for use in protein gel electrophoresis, as well as proteins and protein mol. weight ladders produced by these methods.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:43:37 ON 16 AUG 2005
L1        23701 S THIOREDOXIN
L2        48386 S INCLUSION (A)BOD?
L3          226 S L1 AND L2
L4            2 S "INCLUSION PARTNER PROTEIN"

=> s insoluble
L5       282010 INSOLUBLE

=> s l3 and l5
L6           55 L3 AND L5

=> dup rem l6
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PROCESSING COMPLETED FOR L6
L7 20 DUP REM L6 (35 DUPLICATES REMOVED)

=> d 1-20 ibib ab

L7 ANSWER 1 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
ACCESSION NUMBER: 2004:458566 BIOSIS
DOCUMENT NUMBER: PREV200400458184
TITLE: High-level expression of soluble human beta-defensin-2 in Escherichia coli.
AUTHOR(S): Peng, Li; Xu, Zhinan [Reprint Author]; Fang, Xiangming; Wang, Fang; Cen, Peilin
CORPORATE SOURCE: Inst BioengnDept Chem Engn and Bioengn, Zhejiang Univ, Hangzhou, 310027, China
znxu@zju.edu.cn
SOURCE: Process Biochemistry, (October 29 2004) Vol. 39, No. 12, pp. 2199-2205. print.
ISSN: 1359-5113 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Nov 2004
Last Updated on STN: 24 Nov 2004
AB Human beta-defensin-2 (hBD2) is a short cationic peptide with a broad antimicrobial spectrum. The coding sequence of hBD2 was cloned into pET-32a (+) to construct a fusion expression plasmid, pET32-hBD2, which was transformed into E. coli BL21 (DE3) for expression. The cultivation parameters of the expression vector harboring strain were optimized to produce the fusion protein in soluble form efficiently and to avoid the formation of **insoluble inclusion bodies**.
The optimal conditions were determined as following: cultivation at 28 degreeC in MBL medium, induction at middle stage of exponential growth with 0.8 mM IPTG, and post-induction expression for 8 h. Under the above conditions, a high percentage of the target fusion protein (gtoreq92.3%) was expressed in soluble form and the volumetric productivity of soluble fusion protein reached 1.3 g/l. The culture process was successfully scaled up in a 101 bench-top fermentor. Copyright 2003 Elsevier Ltd. All rights reserved.

L7 ANSWER 2 OF 20 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
ACCESSION NUMBER: 2005:31911 BIOSIS
DOCUMENT NUMBER: PREV200500031767
TITLE: Improving soluble expression of beta-galactosidase in Escherichia coli by fusion with **thioredoxin**.
AUTHOR(S): Nam, E. S.; Jung, H. J.; Ahn, J. K. [Reprint Author]
CORPORATE SOURCE: Dept Agr Sci, Korea Natl Open Univ, Seoul, 110791, South Korea
ajk@knou.ac.kr
SOURCE: Asian-Australasian Journal of Animal Sciences, (December 2004) Vol. 17, No. 12, pp. 1751-1757. print.
ISSN: 1011-2367 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Jan 2005
Last Updated on STN: 12 Jan 2005
AB Recombinant heterologous proteins can be produced as **insoluble** aggregates partially or perfectly inactive in Escherichia coli. One of the strateges to improve the solubility of recombinant proteins is fusion with a partner that is excellent in producing soluble fusion proteins. To improve the production of soluble beta-galactosidase, the gene of Thermus thermophilus KNOUC112 beta-galactosidase (KNOUC112 beta-gal) was fused with **thioredoxin** gene, and optimization of its expression in E.

coli TOP10 was performed. KNOUC112 beta-gal in pET-5b was isolated-out, fused with **thioredoxin** gene in pThioHis C, and transformed to E. coli TOP10. The beta-galactosidase fused with **thioredoxin** was produced in E. coli TOP10 as dinner and trimer. The productivity of fusion beta-galactosidase expressed via pThioHis C at 37degreeC was about 5 times higher than that of unfused beta-galactosidase expressed via pET-5b at 37degreeC. **Inclusion body** of beta-galactosidase was formed highly, regardless of the induction by IPTG when KNOUC112 beta-gal was expressed via pET-5b at 37degreeC. Fusion beta-galactosidase expressed at 37degreeC via pThioHis C without the induction by IPTG was soluble, but the induction by IPTG promoted the formation of **inclusion body**. Lowering the incubation temperature for the expression of fusion gene under 25degreeC prevented the formation of **inclusion body**, optimally at 25degreeC. 0.07 mM of IPTG was sufficient for the soluble expression of fusion gene at 25degreeC. The soluble production of Thermus thermophilus KNOUC112 beta-galactosidase could be increased about 10 times by fusion with **thioredoxin**, and optimization of incubation temperature and IPTG concentration for induction.

L7 ANSWER 3 OF 20 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2004238332 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15135411
TITLE: Expression, purification, and in vitro characterization of recombinant salmon insulin-like growth factor-II.
AUTHOR: Wilkinson Ryan J; Elliott Phillip; Carragher John F; Francis Geoffrey
CORPORATE SOURCE: School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia..
Ryan.Wilkinson@flinders.edu.au
SOURCE: Protein expression and purification, (2004 Jun) 35 (2) 334-43.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200503
ENTRY DATE: Entered STN: 20040512
Last Updated on STN: 20050330
Entered Medline: 20050329

AB The insulin-like growth factors, IGF-I and IGF-II, are single chain polypeptides, which are structurally related to proinsulin and promote proliferation and differentiation of cells in many vertebrate species. Previous attempts to produce recombinant salmon IGF-II (rsIGF-II) were compromised by low expression levels and co-purification of incorrectly cleaved protein with the authentic recombinant product. In this study, a gene containing the coding region for Atlantic salmon (Salmo salar) IGF-II was cloned into a modified pET32a expression vector and transformed into Escherichia coli BL21 trxB (DE3) cells. Upon growth and induction (with IPTG) of the transformant, recombinant salmon IGF-II (rsIGF-II) was expressed as an **insoluble**, 28kDa **thioredoxin**.sIGF-II fusion protein linked by a protease cleavage motif (trx.FAHY.sIGF-II) in **inclusion bodies**. The **inclusion bodies** were subsequently solubilized and the fusion protein was purified by Ni-affinity chromatography. Recombinant IGF-II (7.8kDa) was then released from the fusion partner using H64A subtilisin BPN' protease and purified by reversed-phase HPLC. Homogeneity of the final recombinant product was confirmed by N-terminal amino acid sequencing, ion-spray mass spectrometry, SDS-polyacrylamide gel electrophoresis, and analytical reversed-phase HPLC. The biological activity of rsIGF-II was demonstrated in cultured rat L6 myoblasts and was found to be approximately 9- and 5-fold less potent than recombinant human IGF-I and recombinant salmon

IGF-I, respectively, a result similar to that demonstrated previously with other recombinant fish IGF-II's in non-homologous cell lines.

L7 ANSWER 4 OF 20 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:777839 HCAPLUS
DOCUMENT NUMBER: 139:272056
TITLE: Expression of recombinant mammalian heparin-binding protein (HBP) as fusion protein in Escherichia coli
INVENTOR(S): Woeldike, Helle Fabricius
PATENT ASSIGNEE(S): Leukotech A/S, Den.
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003080660 | A2 | 20031002 | WO 2003-DK207 | 20030326 |
| WO 2003080660 | A3 | 20031218 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 2002-477 A 20020327
AB The present invention relates to methods of making heparin-binding protein (HBP) in a recombinant bacterial expression system. In particular, this invention relates to a method for the preparation of an **insol**. fusion protein comprising heparin-binding protein (HBP), a proteolytic cleavage site, and a second polypeptide in recombinant bacterial cells, said fusion protein being accumulated in **inclusion bodies** in the cytoplasm of bacterium after expression. The invention further relates to methods of separation of the expressed HBP from **inclusion bodies** comprising purification of HBP from the second polypeptide and optionally refolding of said HBP. The invention further features the DNA constructs comprising different HBP-fusion proteins. Invention also relates to use of the HBP-fusion protein produced in bacteria for the production of pure HBP and use of the HBP purified from the fusion protein for the preparation of a medicament. The invention provides protein and cDNA sequence of human and pig heparin-binding protein.

L7 ANSWER 5 OF 20 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003471067 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12963350
TITLE: Expression, purification, and characterization of human enteropeptidase catalytic subunit in Escherichia coli.
AUTHOR: Gasparian Marine E; Ostapchenko Valeriy G; Schulga Alexey A; Dolgikh Dmitry A; Kirpichnikov Mikhail P
CORPORATE SOURCE: Laboratory of Protein Engineering, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, 16/10 Miklukho-Maklaya, 117997 GSP, Moscow, Russia.. marine@nmr.ru
SOURCE: Protein expression and purification, (2003 Sep) 31 (1) 133-9.
Journal code: 9101496. ISSN: 1046-5928.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200406
ENTRY DATE: Entered STN: 20031010
Last Updated on STN: 20040610
Entered Medline: 20040609

AB Enteropeptidase (synonym:enterokinase, EC 3.4.21.9) is a heterodimeric serine protease of the intestinal brush border that activates trypsinogen by highly specific cleavage of the trypsinogen activation peptide following the sequence (Asp)(4)-Lys. The DNA sequence encoding the light chain (catalytic subunit) of human enteropeptidase (GenBank Accession Number U09860) was synthesized from 26 oligonucleotides by polymerase chain reaction and cloned into plasmid pET-32a downstream to the gene of fusion partner **thioredoxin** immediately after the DNA sequence encoding enteropeptidase recognition site. The fusion protein **thioredoxin** /human enteropeptidase light chain was expressed in Escherichia coli BL21(DE3) strain in both soluble and **insoluble** forms. The soluble recombinant fusion protein failed to undergo autocatalytic cleavage and activation; however, autocatalytic cleavage and activation of recombinant human enteropeptidase light chain (L-HEP) were achieved by solubilization and renaturation of the fusion protein from **inclusion bodies** and the active L-HEP was purified on agarose-linked soybean trypsin inhibitor. The purified L-HEP cleaved the synthetic peptide substrate Gly-Asp-Asp-Asp-Asp-Lys-beta-naphthylamide with kinetic parameters K(m)=0.16 mM and k(cat)=115 s(-1) and small ester Z-Lys-SBzl with K(m)=140 microM, k(cat)=133 s(-1). L-HEP associated with soybean trypsin inhibitor slowly and small ester Z-Lys-SBzl cleavage was inhibited with K(i)(*)=2.3 nM. L-HEP digested **thioredoxin**/human epidermal growth factor fusion protein five times faster than equal activity units of bovine recombinant light chain (EKMax, Invitrogen) at the same conditions.

L7 ANSWER 6 OF 20 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:294419 HCAPLUS
DOCUMENT NUMBER: 139:302670
TITLE: Novel approach to obtain biologically active recombinant heterodimeric proteins in Escherichia coli
AUTHOR(S): Austin, Corrine
CORPORATE SOURCE: Unilever R and D Colworth, Sharnbrook, MK44 1LQ, UK
SOURCE: Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2003), 786(1-2), 93-107
CODEN: JCBAAI; ISSN: 1570-0232
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The strategy described in this paper provides a novel approach for recombinant expression of heterodimeric proteins, and is especially suitable for the production of proteins whose characteristics lead to aggregation in E. coli expression systems. Pheromaxein, a steroid-binding protein isolated from boar saliva, is a heterodimeric protein consisting of 10 + 103 rel. mol. mass units (pheromaxein A) and 8 + 103 rel. mol. mass units (pheromaxein C) subunits. Expression of pheromaxein subunits in E. coli resulted in extensive **insol**. aggregation. The difficulty faced in obtaining soluble recombinant pheromaxein subunits was clearly evident when native pheromaxein immediately formed aggregates when it was separated into its individual subunits. An increase in soluble pheromaxein expression in E. coli was obtained when the subunits were expressed as fusion proteins with **thioredoxin**. Pheromaxein genes were inserted sep. into pET32a+ vectors at the NcoI site, resulting in

**thioredoxin**, S·Tag and His·Tag coding regions being upstream of the inserted gene. Soluble pheromaxein A-**thioredoxin** (pheroA/trx) and pheromaxein C-**thioredoxin** (pheroC/trx) fusions were purified to homogeneity, using a laboratory scale S-protein agarose affinity column. Cleavage of **thioredoxin** under normal conditions was not feasible due to the extensive aggregation problems experienced when pheromaxein subunits exist sep. PheroA/trx and pheroC/trx were therefore mixed together and cleaved from **thioredoxin** simultaneously so that pheromaxein subunits were given an instant opportunity to associate under oxido-shuffling conditions. The glutathione oxido-shuffling system allowed the disulfide bridges between pheromaxein A and C to rearrange until the correct native structure was formed. This novel approach combines affinity purification with a coupled fusion protein-cleavage and refolding technique.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 20 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2000200247 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10733885
TITLE: Expression of human cardiac-specific homeobox protein in Escherichia coli.
AUTHOR: Zhao J H; Xu Z; Hua Z C
CORPORATE SOURCE: State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, People's Republic of China.
SOURCE: Protein expression and purification, (2000 Apr) 18 (3) 316-9.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000607

AB Human cardiac-specific homeobox protein cDNA (hCsx) was cloned into expression plasmid pET32a and fused with Escherichia coli **thioredoxin** (Trx). The Trx-Csx fusion protein was under the control of bacteriophage T7 promoter. When expressed in E. coli BL21(DE3), about half of the recombinant Trx-Csx products existed in the form of **insoluble inclusion bodies**. When coexpressed with human protein disulfide isomerase, more than 90% of Trx-Csx products accumulated in the soluble form in the cell lysate. The recombinant Csx fusion protein was purified by one-step metal-chelating affinity chromatography.


L7 ANSWER 8 OF 20 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2000211255 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10744952
TITLE: High-level expression of tetanus toxin fragment C-**thioredoxin** fusion protein in Escherichia coli.
AUTHOR: Ribas A V; Ho P L; Tanizaki M M; Raw I; Nascimento A L
CORPORATE SOURCE: Center of Biotechnology, Instituto Butantan, Av. Vital Brasil, 1500, CEP 05503-900, Sao Paulo, SP, Brazil.
SOURCE: Biotechnology and applied biochemistry, (2000 Apr) 31 ( Pt 2) 91-4.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000606
Last Updated on STN: 20000606
Entered Medline: 20000525

AB An insert of Clostridium tetani DNA corresponding to fragment C of tetanus toxin was amplified by PCR. This 1.4 kb fragment was cloned into the high-expression vector pET32a, under control of the T7 promoter. Expression of this plasmid in Escherichia coli BL21(DE3) resulted in the production of a fusion protein ( approximately 62 kDa) consisting of 112 amino acids of **thioredoxin** and approximately 450 amino acids of fragment C. This fusion protein was recognized by anti-tetanus toxoid antiserum in an ELISA and on immunoblots. The recombinant fragment-C-**thioredoxin** protein was purified significantly in one step by Ni(2+)-chelate Sepharose, the final yield being approximately 35 mg/l. Immunization of animals with the recombinant protein produced antibodies that were able to recognize the tetanus toxin. By using this gene-fusion expression system we produced soluble fragment C of tetanus toxin in a high yield, preventing many problems inherent in the use of other expression systems that produce either **insoluble** fragment C in **inclusion bodies**, or a soluble form, but in low yield, using E. coli as the expression host.

L7 ANSWER 9 OF 20 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:194277 HCAPLUS
DOCUMENT NUMBER: 130:219149
TITLE: Method for producing heterologous proteins as soluble fusion proteins in recombinant cells
INVENTOR(S): Harrison, Roger G.; Davis, Gregory D.
PATENT ASSIGNEE(S): The Board of Regents of the University of Oklahoma, USA
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| WO 9913091 | A1 | 19990318 | WO 1998-US19101 | | 19980914 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | | |
| US 5989868 | A | 19991123 | US 1998-149725 | | 19980908 |
| AU 9894820 | A1 | 19990329 | AU 1998-94820 | | 19980914 |
| US 6207420 | B1 | 20010327 | US 1999-448224 | | 19991123 |
| PRIORITY APPLN. INFO.: | | | US 1997-58698P | P | 19970912 |
| | | | US 1998-88699P | P | 19980609 |
| | | | US 1998-149725 | A | 19980908 |
| | | | US 1998-149725 | A | 19980908 |
| | | | WO 1998-US19101 | W | 19980914 |

AB A fusion protein having a carrier protein which is preferably an E. coli protein having a predicted solubility probability of at least 90 % fused to a target heterologous peptide or protein, and a host cell (especially E. coli) transformed with, or having integrated into its genome, a chimeric gene encoding said fusion protein, are disclosed. An objective of the present

invention is to improve the purification process of recombinant fusion proteins
by avoiding the initial expression of these fusion proteins in E. coli as **insol. inclusion bodies**. The methods and compns. of the present invention permit the production of large amts. of heterologous peptides or proteins in a stable, soluble form in certain host cells which normally express limited amts. of such soluble peptides or proteins. The present invention produces fusion proteins which retain the desirable characteristics of a carrier protein (i.e., stability, solubility, and a high level of expression). Thus, human interleukin 3 was produced in E. coli as a fusion protein with "2X-YgjD" (i.e., the YgjD protein fused to itself), with NusA, with GrpE, with bacterioferritin, and with **thioredoxin**. The fusion proteins were >97, 86, 72, 72, and 54% soluble, resp.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 20 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 2000052947 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10586500
TITLE: Heterologous expression and characterization of endoglucanase I (EGI) from Trichoderma viride HK-75.
AUTHOR: Kwon I; Ekino K; Goto M; Furukawa K
CORPORATE SOURCE: Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan.
SOURCE: Bioscience, biotechnology, and biochemistry, (1999 Oct) 63 (10) 1714-20.
Journal code: 9205717. ISSN: 0916-8451.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000218
Last Updated on STN: 20000218
Entered Medline: 20000207

AB Endoglucanase I (EGI) secreted from Trichoderma viride HK-75 has a unique transglycosylation activity. The genomic and cDNA clones encoding EGI (egl1) of T. viride HK-75 were isolated and characterized. The coding region of egl1, composed of 1392 bp, was found to encode a polypeptide of 464 amino acids that has extensive similarity (93.8%) with EGI of T. reesei. Expression of the egl1 gene in E. coli as a fusion protein (with N-terminal **thioredoxin** and C-terminal histidine tag) led to a large production of a nonglycosylated protein of 62.5 kDa. However, it formed an **insoluble inclusion body**. Upon denaturation with 8 M urea followed by dialysis and successive purification, the enzymatically active recombinant EGI (rEGI) was obtained at a level as high as 18.3 mg/l of 1,000 ml of culture. The rEGI had 67.8% activity for carboxymethyl cellulose (CMC), compared to native EGI (nEGI). The optimum pH and optimum temperature of rEGI were lower than those of nEGI by 0.5 and 5 degrees C, respectively. The rEGI also had narrower CMCase ranges than nEGI in pH and temperature stabilities. However, the catalytic and transglycosylation abilities against cellotriose of rEGI were comparable to those of nEGI. These results suggest that the glycosylation is important for the stabilities of EGI but not critical for the essential enzymatic capacity.

L7 ANSWER 11 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:624940 SCISEARCH
THE GENUINE ARTICLE: 224PT
TITLE: Escherichia coli maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to

which it is fused
AUTHOR: Kapust R B; Waugh D S (Reprint)
CORPORATE SOURCE: NCI, Frederick Canc Res & Dev Ctr, ABL Basic Res Program, POB B, Frederick, MD 21702 USA (Reprint); NCI, Frederick Canc Res & Dev Ctr, ABL Basic Res Program, Frederick, MD 21702 USA
COUNTRY OF AUTHOR: USA
SOURCE: PROTEIN SCIENCE, (AUG 1999) Vol. 8, No. 8, pp. 1668-1674. ISSN: 0961-8368.
PUBLISHER: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Although it is usually possible to achieve a favorable yield of a recombinant protein in Escherichia coli, obtaining the protein in a soluble, biologically active form continues to be a major challenge. Sometimes this problem can be overcome by fusing an aggregation-prone polypeptide to a highly soluble partner. To study this phenomenon in greater detail, we compared the ability of three soluble fusion partners-maltose-binding protein (MBP), glutathione S-transferase; (GST), and **thioredoxin** (TRX)-to inhibit the aggregation of six diverse proteins that normally accumulate in an **insoluble** form. Remarkably, we found that MEP is a far more effective solubilizing agent than the other two fusion partners. Moreover. we demonstrated that in some cases fusion to MEP can promote the proper folding of the attached protein into its biologically active conformation. Thus, MBP seems to be capable of functioning as a general molecular chaperone in the context of a fusion protein. A model is proposed to explain how MBP promotes the solubility and influences the folding of its fusion partners.

L7 ANSWER 12 OF 20 MEDLINE on STN
ACCESSION NUMBER: 1999289510 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10360984
TITLE: Expression of a synthetic gene encoding canine milk lysozyme in Escherichia coli and characterization of the expressed protein.
AUTHOR: Koshiba T; Hayashi T; Miwako I; Kumagai I; Ikura T; Kawano K; Nitta K; Kuwajima K
CORPORATE SOURCE: Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan.
SOURCE: Protein engineering, (1999 May) 12 (5). 429-35. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990730
Last Updated on STN: 19990730
Entered Medline: 19990721

AB A high-expression plasmid of the canine milk lysozyme, which belongs to the family of calcium-binding lysozymes, was constructed in order to study its physico-chemical properties. Because the cDNA sequence of the protein has not yet been determined, a 400 base-pair gene encoding canine milk lysozyme was first designed on the basis of the known amino acid sequence. The gene was constructed by an enzymatic assembly of 21 chemically synthesized oligonucleotides and inserted into an Escherichia coli expression vector by stepwise ligation. The expression plasmid thus

constructed was transformed into BL21(DE3)/pLysS cells. The gene product accumulated as **inclusion bodies** in an **insoluble** fraction. Recombinant canine milk lysozyme was obtained by purification and refolding of the product and showed the same characteristics in terms of bacteriolytic activity and far- and near-UV circular dichroism spectra as the authentic protein. The NMR spectra of refolded lysozyme were also characteristic of a native globular protein. It was concluded that recombinant canine milk lysozyme was folded into the correct native structure. Moreover, the thermal unfolding profiles of the refolded recombinant lysozyme showed a stable equilibrium intermediate, indicating that the molten globule state of this protein was extraordinarily stable. This expression system of canine milk lysozyme will enable biophysical and structural studies of this protein to be extended.

L7 ANSWER 13 OF 20 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1999035447 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9818087
TITLE: Fusion expression of human pro-urokinase with E. coli **thioredoxin**.
AUTHOR: Sun A L; Hua Z C; Yao J; Yang Y H; Yin D Q
CORPORATE SOURCE: Department of Biochemistry, Nanjing University, People's Republic of China.
SOURCE: Biochemistry and molecular biology international, (1998 Oct) 46 (3) 479-86.
Journal code: 9306673. ISSN: 1039-9712.
PUB. COUNTRY: Australia
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 20000303
Entered Medline: 19990122

AB Human pro-urokinase (pro-UK) was cloned into plasmid pET32b and fused to the E. coli **thioredoxin** (trxA). When expressed in E. coli AD494(DE3), the fusion protein Trx-pro-UK accumulated as **insoluble inclusion bodies** and amounted to 35% of total cellular proteins. When co-expressed with molecular chaperones human protein disulfide isomerase (PDI) and E. coli GroESL, all the expressed products still existed in the form of **insoluble inclusion bodies**.

L7 ANSWER 14 OF 20 NTIS COPYRIGHT 2005 NTIS on STN
ACCESSION NUMBER: 1997(18):05240
NTIS ORDER NUMBER: AD-A325 566/8/XAB
TITLE: Investigation of the Solubility and Enzymatic Activity of a **Thioredoxin**-Gelonin Fusion Protein. Master's thesis.
AUTHOR: Licata, M. J.
CORPORATE SOURCE: Texas Univ. at Austin. (043127000 347800)
NUMBER OF REPORT: AD-A325 566/8/XAB
96p; May 1997
CONTROLLED TERM: Dissertation
COUNTRY: United States
LANGUAGE: English
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22161, USA.
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AB A synthetic gene for the ribosome-inactivating protein (RIP), gelonin, was previously engineered and inserted into the pET-21a plasmid under the control of the T7 promoter by researchers at M.D. Anderson Cancer Research Institute in Houston, Texas. Upon induction of Escherichia coli (E. coli) strain BL-21(DE3)pLysS containing this pET-2 1 a/gel plasmid, the resulting gelonin protein forms **insoluble** aggregates, known as **inclusion bodies**, and exhibits no activity under the assay conditions tested. By genetically fusing gelonin to the highly stable and soluble protein, **thioredoxin**, it was thought that there would be an increase in the solubility of gelonin, possibly accompanied by a measurable amount of enzymatic activity.

L7 ANSWER 15 OF 20 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 97325530 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9181583
TITLE: Manipulating the aggregation and oxidation of human SPARC in the cytoplasm of Escherichia coli.
AUTHOR: Schneider E L; Thomas J G; Bassuk J A; Sage E H; Baneyx F
CORPORATE SOURCE: Department of Chemical Engineering, University of Washington, Seattle 98195, USA.
CONTRACT NUMBER: GM40711 (NIGMS)
P50-DK-47659 (NIDDK)
SOURCE: Nature biotechnology, (1997 Jun) 15 (6) 581-5.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 20000303
Entered Medline: 19970819

AB Human SPARC (secreted protein acidic and rich in cysteine), an extracellular matrix protein containing 14 cysteine residues, was found to partition equally between soluble and **insoluble** cellular fractions when overexpressed in the Escherichia coli cytoplasm. While the growth temperature and medium pH had little effect on **inclusion body** formation, co-overproduction of the dnaKJ operon, but not of the groE operon, suppressed aggregation at the expense of intracellular accumulation. Although both forms of the protein were fully reduced in wild-type cells, 70% to 85% of soluble and **insoluble** SPARC could be converted into oxidized species in a **thioredoxin** reductase (trxB) null mutant following incubation on ice. Approximately 15% to 20% of SPARC exhibited the electrophoretic mobility of the biologically active protein. Overproduction of the dnaKJ operon in trxB cells decreased the formation of disulfide-bonded SPARC multimers in the aggregated material but not in its soluble counterpart. Our results suggest that the activity responsible for disulfide bond formation in trxB mutants acts at the post-translational level and is able to freely diffuse within **inclusion bodies**.

L7 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:230242 HCAPLUS
TITLE: Aggregation and oxidation of sparc in the cytoplasm of wild type and trxB E. coli.
AUTHOR(S): Baneyx, Francois; Schneider, Eleonor L.; Thomas, Jeffrey G.; Bassuk, James A.
CORPORATE SOURCE: Dept. Chemical Engineering, University Washington, Seattle, WA, 98195, USA
SOURCE: Book of Abstracts, 213th ACS National Meeting, San

Francisco, April 13-17 (1997), BIOC-176. American Chemical Society: Washington, D. C.
CODEN: 64AOAA
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Human SPARC, an extracellular matrix protein containing 14 cysteine residues, was found to partition equally between soluble and **insol**. cellular fractions when overexpressed in the E. coli cytoplasm. While the growth temperature and medium pH had little effect on **inclusion body** formation, co-overprodn. of the dnaKJ operon, but not of the groE operon, suppressed aggregation at the expense of intracellular accumulation. Although both forms of the protein were fully reduced in wild type cells, 70-85% of soluble and **insol**. SPARC could be converted into oxidized species in a **thioredoxin** reductase (trxB) null mutant following incubation on ice. Approx. 15-20% of SPARC exhibited the electrophoretic mobility of the biol. active protein. Overprodn. of the dnaKJ operon in trxB cells decreased the formation of disulfide-bonded SPARC multimers in the aggregated material, but not in its soluble counterpart. Our results suggest that the activity responsible for disulfide bond formation in trxB mutants acts at the post-translational level and is able to freely diffuse within **inclusion bodies**.

L7 ANSWER 17 OF 20 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1997-12126 BIOTECHDS
TITLE: Aggregation and oxidation of SPARC in the cytoplasm of wild-type and trxB Escherichia coli; human recombinant extracellular matrix protein expression in mutant bacterium **inclusion body** (conference abstract)
AUTHOR: Baneyx F; Schneider E L; Thomas J G; Bassuk J A
CORPORATE SOURCE: Univ.Washington-Seattle
LOCATION: Depts. of Chemical Engineering and Biological Structure, Box 351750, Seattle, WA 98195, USA.
SOURCE: Abstr.Pap.Am.Chem.Soc.; (1997) 213 Meet., Pt.1, BIOT176
CODEN: ACSRAL
ISSN: 0065-7727
American Chemical Society, 213th ACS National Meeting, San Francisco, CA, 13-17 April, 1997.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human SPARC, an extracellular matrix protein containing 14 cysteine residues, was found to partition equally between soluble and **insoluble** cellular fractions when overexpressed in the Escherichia coli cytoplasm. While the growth temperature and the medium pH had little effect on **inclusion body** formation, co-overproduction of the dnaKJ operon, but not of the groE operon, suppressed aggregation at the expense of intracellular accumulation. Although both forms of the protein were fully reduced in wild type cells, 70-85% of SPARC could be converted into oxidized species in a **thioredoxin**-reductase (trxB, EC-1.6.4.5) null mutant following incubation on ice. Approximately 15-20% of SPARC exhibited the electrophoretic mobility of the biologically active protein. Overproduction of the dnaKJ operon in trxB cells decreased the formation of disulfide-bonded SPARC multimers in the aggregated material, but not in its soluble counterpart. The activity responsible for disulfide bond formation in trxB mutants may act at the post-translational level and may be able to freely diffuse within **inclusion bodies**. (0 ref)

L7 ANSWER 18 OF 20 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:728859 SCISEARCH
THE GENUINE ARTICLE: TB466
TITLE: INCREASE OF SOLUBILITY OF FOREIGN PROTEINS IN ESCHERICHIA-COLI BY COPRODUCTION OF THE BACTERIAL **THIOREDOXIN**
AUTHOR: YASUKAWA T (Reprint); KANEIISHII C; MAEKAWA T; FUJIMOTO J; YAMAMOTO T; ISHII S
CORPORATE SOURCE: INST PHYS & CHEM RES, MOLEC GENET LAB, TSUKUBA, IBARAKI 305, JAPAN; UNIV TSUKUBA, INST MED SCI, TSUKUBA, IBARAKI 305, JAPAN; UNIV TOKYO, INST MED SCI, DEPT ONCOL, MINATO KU, TOKYO 108, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 OCT 1995) Vol. 270, No. 43, pp. 25328-25331.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 27
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995
*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Eukaryotic proteins are frequently produced in Escherichia coli as **insoluble** aggregates. This is one of the barriers to studies of macromolecular structure. We have examined the effect of coproduction of the E. coli **thioredoxin** (Trx) or E. coli chaperones GroESL on the solubility of various foreign proteins. The solubilities of all eight vertebrate proteins examined including transcription factors and kinases were increased dramatically by coproduction of Trx. Overproduction of E. coli chaperones GroESL increased the solubilities of four out of eight proteins examined. Although the tyrosine kinase Lck that was produced as an **insoluble** form and solubilized by urea treatment had a very low autophosphorylating activity, Lck produced in soluble form by coproduction of Trx had an efficient activity. These results suggest that the proteins produced in soluble form by coproduction of Trx have the native protein conformation. The mechanism by which coproduction of Trx increases the solubility of the foreign proteins is discussed.

L7 ANSWER 19 OF 20 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1993-12568 BIOTECHDS
TITLE: Escherichia coli as a host system for gene expression and the formation of **inclusion bodies**;
avoidance of **inclusion body** formation by recombinant **thioredoxin** fusion protein production for e.g. interleukin or macrophage colony stimulating factor production
AUTHOR: Mazza P
LOCATION: Societa Editoriale Farmaceutica, Via Ausonio 12, Milan 20132, Italy.
SOURCE: Boll.Chim.Farm.; (1993) 132, 7, 247-51
CODEN: BCFAAI
DOCUMENT TYPE: Journal
LANGUAGE: Italian

AB The use of Escherichia coli as a host system for gene cloning is discussed. The formation of **inclusion bodies** is the most important factor limiting application of this microorganism to the production of human proteins of pharmaceutical interest. In E. coli with a cloned gene under the control of efficient expression sequences it is possible to achieve very high levels of production, where the exogenous protein represents up to 30-40% of the total cellular proteins. However, under such conditions, **inclusion bodies** are formed as

**insoluble** protein aggregates in the cytoplasm. The formation of **inclusion bodies** does not depend upon the dimensions of the protein, and rarely depends upon the promotor or expression level, but is strongly influenced by the host strain and the growth conditions. A strategy based on gene fusion with **thioredoxin** has resulted in high levels of production of soluble eukaryotic proteins, e.g. interleukin-1 to -6 and macrophage colony stimulating factor, in E. coli. The **thioredoxin** of E. coli is extremely thermostable, allowing protein purification by heat treatment. (5 ref)

L7 ANSWER 20 OF 20 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 93152129 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7763371
TITLE: A **thioredoxin** gene fusion expression system that circumvents **inclusion body** formation in the E. coli cytoplasm.
AUTHOR: LaVallie E R; DiBlasio E A; Kovacic S; Grant K L; Schendel P F; McCoy J M
CORPORATE SOURCE: Genetics Institute, Cambridge, MA 02140.
SOURCE: Bio/technology (Nature Publishing Company), (1993 Feb) 11 (2) 187-93.
Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 19950809
Entered Medline: 19930309

AB We have developed a versatile Escherichia coli expression system based on the use of E. coli **thioredoxin** (trxA) as a gene fusion partner. The broad utility of the system is illustrated by the production of a variety of mammalian cytokines and growth factors as **thioredoxin** fusion proteins. Although many of these cytokines previously have been produced in E. coli as **insoluble** aggregates or " **inclusion bodies**", we show here that as **thioredoxin** fusions they can be made in soluble forms that are biologically active. In general we find that linkage to **thioredoxin** dramatically increases the solubility of heterologous proteins synthesized in the E. coli cytoplasm, and that **thioredoxin** fusion proteins usually accumulate to high levels. Two additional properties of E. coli **thioredoxin**, its ability to be specifically released from the E. coli cytoplasm by osmotic shock or freeze/thaw treatments and its intrinsic thermal stability, are retained by some fusions and provide convenient purification steps. We also find that the active-site loop of E. coli **thioredoxin** can be used as a general site for small peptide insertions, allowing for the high level production of soluble peptides in the E. coli cytoplasm.

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=> e chatterjee d/au
E1          12     CHATTERJEE CHIRADIP/AU
E2          43     CHATTERJEE CHITRALEKHA/AU
E3        1046 --> CHATTERJEE D/AU
E4           2     CHATTERJEE D */AU
E5           1     CHATTERJEE D B/AU
E6           6     CHATTERJEE D C/AU
E7           5     CHATTERJEE D D/AU
E8           1     CHATTERJEE D E B/AU
E9          36     CHATTERJEE D J/AU
E10        380     CHATTERJEE D K/AU
E11         54     CHATTERJEE D N/AU
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E12          23      CHATTERJEE D P/AU

=> s e3
L8         1046 "CHATTERJEE D"/AU

=> e longo m/au
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E2            6      LONGO LUIZ S JR/AU
E3          646 --> LONGO M/AU
E4          100      LONGO M A/AU
E5            9      LONGO M B/AU
E6           23      LONGO M C/AU
E7            2      LONGO M CASTELLANI/AU
E8           14      LONGO M D/AU
E9            1      LONGO M D C/AU
E10          17      LONGO M F/AU
E11          17      LONGO M G/AU
E12          15      LONGO M I/AU

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'E3' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):s e3
'S' IS NOT A VALID FORMAT
'E3' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> s e3
L9           646 "LONGO M"/AU

=> e flynn e/au
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E2            3      FLYNN DUSTIE/AU
E3          272 --> FLYNN E/AU
E4           53      FLYNN E A/AU
E5            1      FLYNN E B/AU
E6            5      FLYNN E C/AU
E7            9      FLYNN E D/AU
E8            3      FLYNN E E/AU
E9            1      FLYNN E F/AU
E10          11      FLYNN E H/AU
E11         151      FLYNN E J/AU
E12           1      FLYNN E J */AU

=> s e3
L10          272 "FLYNN E"/AU

=> e oberfelder r/au
E1            1      OBERFELD SHELDON M/AU
E2            1      OBERFELDARZT/AU
E3           14 --> OBERFELDER R/AU
E4           30      OBERFELDER R W/AU
E5            6      OBERFELDER ROBERT/AU
E6            5      OBERFELDER ROBERT W/AU
E7            1      OBERFELDER ROBERT WILLIAM/AU
E8           15      OBERFELL C R/AU
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E9          10     OBERFELL CHARLES R/AU
E10          1     OBERFELL CHAS R/AU
E11         73     OBERFELL G G/AU
E12          1     OBERFELL G S/AU

=> s e3-e7
L11         56 ("OBERFELDER R"/AU OR "OBERFELDER R W"/AU OR "OBERFELDER ROBERT"
               /AU OR "OBERFELDER ROBERT W"/AU OR "OBERFELDER ROBERT WILLIAM"/A
               U)

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     (FILE 'HOME' ENTERED AT 11:43:12 ON 16 AUG 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:43:37 ON 16 AUG 2005
L1       23701 S THIOREDOXIN
L2       48386 S INCLUSION (A)BOD?
L3         226 S L1 AND L2
L4           2 S "INCLUSION PARTNER PROTEIN"
L5      282010 S INSOLUBLE
L6          55 S L3 AND L5
L7          20 DUP REM L6 (35 DUPLICATES REMOVED)
               E CHATTERJEE D/AU
L8        1046 S E3
               E LONGO M/AU
L9         646 S E3
               E FLYNN E/AU
L10        272 S E3
               E OBERFELDER R/AU
L11         56 S E3-E7

=> s l8 or l9 or l10 or l11
L12      2013 L8 OR L9 OR L10 OR L11

=> s l3 and l12
L13         4 L3 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14          3 DUP REM L13 (1 DUPLICATE REMOVED)

=> d 1-3 ibib ab

L14  ANSWER 1 OF 3  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
ACCESSION NUMBER:   2004:219988  BIOSIS
DOCUMENT NUMBER:    PREV200400223055
TITLE:              Methods for production of proteins.
AUTHOR(S):          Chatterjee, Deb [Inventor, Reprint Author]; Longo, Mary
                    [Inventor]; Flynn, Elizabeth [Inventor]; Oberfelder,
                    Robert [Inventor]
CORPORATE SOURCE:   North Potomac, MD, USA
                    ASSIGNEE: Invitrogen Corporation, Frankfurt am Main, DE,
                    USA
PATENT INFORMATION: US 6703484 20040309
SOURCE:             Official Gazette of the United States Patent and Trademark
                    Office Patents, (Mar 9 2004) Vol. 1280, No. 2.
                    http://www.uspto.gov/web/menu/patdata.html. e-file.
                    ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE:      Patent
LANGUAGE:           English
ENTRY DATE:         Entered STN: 21 Apr 2004
                    Last Updated on STN: 21 Apr 2004
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AB The current invention provides methods for producing a polypeptide as **inclusion bodies** in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an inclusion partner protein, such as E. coli **thioredoxin** or a modified E. coli **thioredoxin**, such that host cells comprising the gene construct produce the polypeptide as intracellular **inclusion bodies**. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The invention further provides methods for producing protein molecular weight ladders for use in protein gel electrophoresis, as well as proteins and protein molecular weight ladders produced by these methods.

L14 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 1998-09126 BIOTECHDS
TITLE: Production of polypeptides as **inclusion bodies**;
recombinant protein preparation by plasmid pTrc99A or plasmid pTrxfus vector-mediated **thioredoxin** expression in Escherichia coli **inclusion body**
AUTHOR: **Chatterjee D**; **Longo M**; **Flynn E**; **Oberfelder R**
PATENT ASSIGNEE: Life-Technol.
LOCATION: Rockville, MD, USA.
PATENT INFO: WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO: US 1997-34658 8 Jan 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-399134 [34]
AB A new method for the preparation of a protein in the form of an **inclusion body** involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner ( **thioredoxin** or modified **thioredoxin**), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as **inclusion bodies**. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or **thioredoxin**. (84pp)

L14 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:421271 BIOSIS
DOCUMENT NUMBER: PREV199799720474
TITLE: Protein expression by inclusion.
AUTHOR(S): **Oberfelder, R. W.**; **Flynn, E.**;

**Chatterjee, D.**

SOURCE: FASEB Journal, (1997) Vol. 11, No. 9, pp. A1200.
Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology. San Francisco, California, USA. August 24-29, 1997.
CODEN: FAJOEC. ISSN: 0892-6638.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 8 Oct 1997

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 11:43:37 ON 16 AUG 2005

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| L1 | 23701 | S | THIOREDOXIN |
| L2 | 48386 | S | INCLUSION (A)BOD? |
| L3 | 226 | S | L1 AND L2 |
| L4 | 2 | S | "INCLUSION PARTNER PROTEIN" |
| L5 | 282010 | S | INSOLUBLE |
| L6 | 55 | S | L3 AND L5 |
| L7 | 20 | DUP REM | L6 (35 DUPLICATES REMOVED) |
| | | E | CHATTERJEE D/AU |
| L8 | 1046 | S | E3 |
| | | E | LONGO M/AU |
| L9 | 646 | S | E3 |
| | | E | FLYNN E/AU |
| L10 | 272 | S | E3 |
| | | E | OBERFELDER R/AU |
| L11 | 56 | S | E3-E7 |
| L12 | 2013 | S | L8 OR L9 OR L10 OR L11 |
| L13 | 4 | S | L3 AND L12 |
| L14 | 3 | DUP REM | L13 (1 DUPLICATE REMOVED) |

10/728,766

| | L # | Hits | Search Text |
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| 1 | L1 | 1 | "6703484".pn. |
| 2 | L2 | 3 | "inclusion partner protein" |
| 3 | L3 | 1 | l1 and l2 |
| 4 | L4 | 5189 | thioredoxin |
| 5 | L5 | 2 | incusion adj bod$3 |
| 6 | L6 | 6782 | inclusion adj bod$3 |
| 7 | L7 | 660 | l4 and l6 |
| 8 | L8 | 340681 | fus$3 |
| 9 | L9 | 640 | l7 and l8 |
| 10 | L10 | 31747 | CHATTERJEE LONGO FLYNN OBERFELDER |
| 11 | L11 | 41 | l7 and l10 |

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| 1 | 20050811 | 8 | US 20050175582 A1 | Non-antigenic toxin-conjugate and fusion protein of internalizing receptor system |
| 2 | 20050721 | 72 | US 20050159590 A1 | Variants of interleukin-1 receptor antagonist: compositions and uses thereof |
| 3 | 20050714 | 41 | US 20050153327 A1 | Human ataxin-1-like polypeptide IMX97018 |
| 4 | 20050707 | 238 | US 20050150000 A1 | Nucleic acid sequences encoding type III tenebrio antifreeze proteins and method for assaying activity |
| 5 | 20050707 | 21 | US 20050148037 A1 | Method for examining WT1-related disease |
| 6 | 20050707 | 273 | US 20050148016 A1 | Novel human G-protein coupled receptor, HGPRBMY29sv1 polypeptides |
| 7 | 20050630 | 77 | US 20050143386 A1 | Diagnosis and management of infection caused by Chlamydia |
| 8 | 20050630 | 86 | US 20050142620 A1 | Compositions and methods for the therapy and diagnosis of lung cancer |
| 9 | 20050616 | 351 | US 20050130286 A1 | POLYNUCLEOTIDES ENCODING NOVEL HUMAN PHOSPHATASES |
| 10 | 20050616 | 37 | US 20050130269 A1 | Fusion proteins |
| 11 | 20050616 | 34 | US 20050130259 A1 | Expression vector, host, fused protein, process for producing fused protein and process for producing protein |

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| 12 | 20050609 | 20 | US 20050124790 A1 | Process for preparation of polypeptides of interest from fusion polypeptides |

| | Issue Date | Pages | Document ID | Title |
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| 13 | 20050609 | 31 | US 20050124064 A1 | Antimicrobial polypeptides from pseudoplectania nigrella |
| 14 | 20050602 | 25 | US 20050119462 A1 | Expression vectors and promoters for heterologous gene expression |
| 15 | 20050602 | 49 | US 20050118729 A1 | Microarrays of cellulose binding chimeric proteins and methods of use thereof |
| 16 | 20050602 | 156 | US 20050118632 A1 | Polynucleotides and polypeptides encoding a novel metalloprotease, Protease-40b |
| 17 | 20050526 | 66 | US 20050112706 A1 | Diagnostic and prognostic methods for prostate cancers |
| 18 | 20050519 | 34 | US 20050106681 A1 | Enhanced solubility of recombinant proteins |
| 19 | 20050519 | 126 | US 20050106597 A1 | Staphylococcus aureus polynucleotides and polypeptides |
| 20 | 20050505 | 82 | US 20050095592 A1 | Identification of ovarian cancer tumor markers and therapeutic targets |
| 21 | 20050428 | 27 | US 20050089956 A1 | Process for producing alpha 2,3/ alpha 2,8-sialyltransferase and sialic acid-containing complex sugar |
| 22 | 20050421 | 32 | US 20050085624 A1 | Method and device |
| 23 | 20050414 | 106 | US 20050080232 A1 | Alzheimer's disease secretase, app substrates therefor, and uses therefor |
| 24 | 20050414 | 58 | US 20050079575 A1 | Human receptor molecules |

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| 25 | 20050407 | 64 | US 20050076407 A1 | Plant defensin polynucleotides and methods of use thereof |

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|---|---|---|---|---|
| 26 | 20050407 | 45 | US 20050074853 A1 | Recombinant MHC molecules useful for manipulation of antigen-specific T-cells |
| 27 | 20050407 | 76 | US 20050074851 A1 | Vesicle associated proteins |
| 28 | 20050407 | 51 | US 20050074756 A1 | FALP proteins |
| 29 | 20050317 | 74 | US 20050059109 A1 | Methods and compositions for polypeptide engineering |
| 30 | 20050317 | 123 | US 20050059070 A1 | Human single nucleotide polymorphisms in ION channels and other proteins |
| 31 | 20050303 | 157 | US 20050048620 A1 | Polynucleotides encoding a novel human neuronal cell adhesion protein, BGS-28, and variants thereof |
| 32 | 20050303 | 132 | US 20050048610 A1 | Human transport proteins |
| 33 | 20050303 | 102 | US 20050048572 A1 | Methods and compositions for increasing antibody production |
| 34 | 20050224 | 79 | US 20050042738 A1 | Carbohydrate-associated proteins |
| 35 | 20050224 | 55 | US 20050042690 A1 | Diagnosis and management of infection caused by chlamydia |
| 36 | 20050224 | 291 | US 20050042623 A1 | Systems for capture and analysis of biological particles and methods using the systems |
| 37 | 20050210 | 169 | US 20050033018 A1 | Receptors and membrane-associated proteins |
| 38 | 20050210 | 258 | US 20050032166 A1 | Polynucleotides encoding novel adiponectin receptor variants |

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| 39 | 20050210 | 398 | US 20050031634 A1 | Compositions and methods for the therapy and diagnosis of ovarian cancer |
| 40 | 20050203 | 88 | US 20050026256 A1 | Alzheimer's disease secretase |
| 41 | 20050203 | 266 | US 20050026191 A1 | Polynucleotides encoding novel guanylate binding proteins (GBP's) |
| 42 | 20050127 | 29 | US 20050020528 A1 | Chemokines with amino-terminal modifications |
| 43 | 20050127 | 39 | US 20050019829 A1 | Protein/solubility folding assessed by structural complementation |
| 44 | 20050127 | 37 | US 20050019815 A1 | Mammalian selenoprotein differentially expressed in tumor cells |
| 45 | 20050120 | 79 | US 20050014224 A1 | GITR ligand and GITR ligand-related molecules and antibodies and uses thereof |
| 46 | 20050120 | 148 | US 20050014177 A1 | Human sterol response element binding protein 1 (SREBP1) single nucleotide polymorphisms |
| 47 | 20050120 | 225 | US 20050013825 A1 | Vaccine containing the catalytic subunit of telomerase for treating cancer |
| 48 | 20050106 | 15 | US 20050003478 A1 | Process for producing beta-1, 3-n-acetylglucosamine transferase and n-acetylglucosamine-containing composite saccharide |
| 49 | 20050106 | 70 | US 20050003447 A1 | Human cytoskeleton associated proteins |

| | Issue Date | Pages | Document ID | Title |
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| 50 | 20050106 | 71 | US 20050003416 A1 | Novel modified corin molecules having substitute activation sequences and uses thereof |
| 51 | 20041230 | 91 | US 20040268425 A1 | Soluble hyaluronidase glycoprotein (sHASEGP), process for preparing the same, uses and pharmaceutical compositions comprising thereof |
| 52 | 20041230 | 167 | US 20040265890 A1 | Novel human leucine-rich repeat containing protein expressed predominately in small intestine, HLRRSI1 |
| 53 | 20041223 | 36 | US 20040258694 A1 | Hepatitis C receptor protein CD81 |
| 54 | 20041216 | 131 | US 20040254350 A1 | Immune response associated proteins |
| 55 | 20041216 | 117 | US 20040253701 A1 | Protein and peptide fragments from mouse telomerase reverse transcriptase |
| 56 | 20041216 | 16 | US 20040253670 A1 | Process for producing alpha1,4-galactosyltransferase and galactose-containing complex sugar |
| 57 | 20041216 | 82 | US 20040253578 A1 | Dynamic action reference tools |
| 58 | 20041209 | 67 | US 20040248253 A1 | Methods and compositions for polypeptide engineering |
| 59 | 20041209 | 144 | US 20040248251 A1 | Receptors and membrane associated proteins |
| 60 | 20041209 | 225 | US 20040247613 A1 | Treating cancer using a telomerase vaccine |

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| 61 | 20041202 | 223 | US 20040242529 A1 | Vector encoding inactivated telomerase for treating cancer |

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| 62 | 20041202 | 271 | US 20040241748 A1 | Self-assembling arrays and uses thereof |
| 63 | 20041125 | 54 | US 20040236072 A1 | Surface proteins of streptococcus pyogenes |
| 64 | 20041125 | 89 | US 20040235721 A1 | Recombinant proteins of parapoxvirus ovis and pharmaceutical compositions therefrom |
| 65 | 20041125 | 86 | US 20040235072 A1 | Compositions and methods for the therapy and diagnosis of lung cancer |
| 66 | 20041125 | 107 | US 20040234976 A1 | Alzheimer's disease secretase, app substrates therefor, and uses therefor |
| 67 | 20041118 | 361 | US 20040229315 A1 | Polynucleotides encoding novel variants of the TRP channel family member, LTRPC3 |
| 68 | 20041118 | 146 | US 20040229262 A1 | Polynucleotide encoding a novel human P2X7 splice variant, HBMYP2X7v |
| 69 | 20041111 | 171 | US 20040224911 A1 | Transporters and ion channels |
| 70 | 20041111 | 186 | US 20040224408 A1 | THAP proteins as nuclear receptors for chemokines and roles in transcriptional regulation, cell proliferation and cell differentiation |
| 71 | 20041104 | 35 | US 20040219667 A1 | Monoclonal antibody for human telomerase catalytic subunit |
| 72 | 20041104 | 15 | US 20040219594 A1 | Esterase, its DNA, its overexpression and production of optically active aryl propionic acids using the same |
| 73 | 20041104 | 17 | US 20040219553 A1 | Alpha-1,2-fucosyltransferase and dna encoding the same |

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| 74 | 20041104 | 44 | US 20040219529 A1 | Compositions and methods of using a synthetic DNase I |
| 75 | 20041104 | 109 | US 20040219525 A1 | Plant polynucleotides encoding novel prenyl proteases |
| 76 | 20041028 | 76 | US 20040214277 A1 | Methods and compositions for polypeptide engineering |
| 77 | 20041028 | 84 | US 20040214258 A1 | Covalent tethering of functional groups to proteins |
| 78 | 20041021 | 29 | US 20040209323 A1 | Protein expression by codon harmonization and translational attenuation |
| 79 | 20041021 | 293 | US 20040209282 A1 | Methods for producing polypeptide-tagged collections and capture systems containing the tagged polypeptides |
| 80 | 20041014 | 155 | US 20040204576 A1 | Polynucleotides encoding a novel human phosphatase, BMY_HPP13 |
| 81 | 20041014 | 31 | US 20040204563 A1 | Methods for production of proteins |
| 82 | 20041014 | 141 | US 20040203014 A1 | Neurotransmisson-associated proteins |
| 83 | 20040930 | 112 | US 20040191843 A1 | Cell-killing molecules and methods of use thereof |
| 84 | 20040909 | 29 | US 20040175825 A1 | Monoclonal antibody binding to mt4-mmp catalytic domain |
| 85 | 20040902 | 199 | US 20040171131 A1 | Polynucleotides encoding a novel testis-specific tubulin tyrosine-ligase-like protein, BGS42 |
| 86 | 20040902 | 47 | US 20040171112 A1 | Oxidation reduction sensitive green fluorescent protein variants |

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| 87 | 20040902 | 32 | US 20040170976 A1 | Solubility reporter gene constructs |
| 88 | 20040826 | 75 | US 20040167060 A1 | Inhibitor and stimulator of stem cell proliferation and uses thereof |
| 89 | 20040826 | 27 | US 20040166565 A1 | Recombinant proteins containing Shiga-like toxin and vascular endothelial growth factor fragments |
| 90 | 20040826 | 107 | US 20040166507 A1 | Alzheimer's disease secretase, app substrates therefor, and uses therefor |
| 91 | 20040826 | 175 | US 20040166501 A1 | Receptors and membrane-associated proteins |
| 92 | 20040819 | 13 | US 20040161832 A9 | Esterase, its DNA, its overexpression and production of optically active aryl propionic acids using the same |
| 93 | 20040819 | 40 | US 20040161822 A1 | Protein having antithrombotic activity and method for producing the same |
| 94 | 20040812 | 60 | US 20040157771 A1 | Rank-ligand-induced sodium/proton antiporter polypeptides |
| 95 | 20040812 | 31 | US 20040157291 A1 | Generation of specific binding partners binding to (poly)peptides encoded by genomic DNA fragments or ESTs |
| 96 | 20040812 | 171 | US 20040157234 A1 | Polynucleotides encoding a novel testis-specific tubulin tyrosine-ligase-like protein, BGS42 |
| 97 | 20040805 | 72 | US 20040152874 A1 | Transporter and ion channels |

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| 98 | 20040729 | 20 | US 20040146969 A1 | Process for producing recombinant protein and fused protein |

| | Issue Date | Pages | Document ID | Title |
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| 99 | 20040729 | 21 | US 20040146923 A1 | Tendon-inducing compositions |
| 100 | 20040722 | 19 | US 20040142414 A1 | Production of heterologous protein in a minimal culture medium |
| 101 | 20040722 | 44 | US 20040142361 A1 | Compositions and methods for the therapy and diagnosis of breast cancer |
| 102 | 20040708 | 109 | US 20040132156 A1 | Modified hepsin molecules having a substitute activation sequence and uses thereof |
| 103 | 20040701 | 23 | US 20040128706 A1 | Method for screening for anti-amyloidogenic properties and method for treatment of neurodegenerative disease |
| 104 | 20040701 | 46 | US 20040127683 A1 | Transporters and ion channels |
| 105 | 20040701 | 233 | US 20040126362 A1 | Compositions and methods for WT1 specific immunotherapy |
| 106 | 20040617 | 140 | US 20040116666 A1 | Transporters and ion channels |
| 107 | 20040617 | 32 | US 20040116662 A1 | Antigenic fragment of human T-lymphotropic virus |
| 108 | 20040610 | 28 | US 20040110260 A1 | Recombinant minimal catalytic vanadium haloperoxidases and their uses |
| 109 | 20040610 | 124 | US 20040109853 A1 | Biological active coating components, coatings, and coated surfaces |
| 110 | 20040603 | 93 | US 20040106125 A1 | Neurotransmission-associated proteins |

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| 111 | 20040520 | 81 | US 20040097711 A1 | Immunoglobulin superfamily proteins |

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| 112 | 20040506 | 474 | US 20040086896 A1 | Polynucleotides and polypeptides associated with the NF-kB pathway |
| 113 | 20040506 | 97 | US 20040086881 A1 | Novel human G-protein coupled receptor, BMSOTR, and splice variant thereof |
| 114 | 20040429 | 131 | US 20040082002 A1 | 37 staphylococcus aureus genes and polypeptides |
| 115 | 20040422 | 48 | US 20040077837 A1 | Microtubule-associated proteins and tubulins |
| 116 | 20040422 | 40 | US 20040077066 A1 | Purified active HCV NS2/3 protease |
| 117 | 20040422 | 31 | US 20040076606 A1 | Methods of modulating inflammation by administration of interleukin-19 and inhibitors of IL-19 binding |
| 118 | 20040415 | 216 | US 20040073016 A1 | Compositions and methods for the therapy and diagnosis of breast cancer |
| 119 | 20040415 | 64 | US 20040072740 A1 | Directed evolution of enzymes and antibodies |
| 120 | 20040415 | 22 | US 20040072179 A1 | Mutant proteinase with reduced self-cleavage activity and method of purification |
| 121 | 20040408 | 295 | US 20040068096 A1 | Human single nucleotide polymorphisms in organic anion transport and multi-drug resistant proteins |
| 122 | 20040318 | 49 | US 20040053834 A1 | Enzymes involved in glycoprotein and glycolipid metabolism |
| 123 | 20040318 | 42 | US 20040053425 A1 | Quantitative measurement of proteins using genetically-engineeredglucose oxidase fusion molecules |

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| 124 | 20040318 | 182 | US 20040053258 A1 | Transporters and ion channels |
| 125 | 20040318 | 88 | US 20040052810 A1 | Abrogen polypeptides, nucleic acids encoding them and methods for using them to inhibit angiogenesis |
| 126 | 20040318 | 108 | US 20040052777 A1 | Kringle polypeptides and methods for using them to inhibit angiogenesis |
| 127 | 20040311 | 105 | US 20040049010 A1 | Transmembrane proteins |
| 128 | 20040311 | 148 | US 20040048311 A1 | Use of collections of binding sites for sample profiling and other applications |
| 129 | 20040311 | 107 | US 20040048303 A1 | Alzheimer's disease secretase, APP substrates therefor, and uses therefor |
| 130 | 20040311 | 204 | US 20040048302 A1 | Novel metalloprotease polypeptide, MP-1 |
| 131 | 20040304 | 67 | US 20040043424 A1 | Immunoglobulin superfamily proteins |
| 132 | 20040304 | 108 | US 20040043408 A1 | Alzheimer's disease secretase, APP substrates therefor, and uses therefor |
| 133 | 20040304 | 179 | US 20040043407 A1 | Polynucleotides encoding a novel metalloprotease, MP-1 |
| 134 | 20040226 | 85 | US 20040040054 A1 | Plant polynucleotides encoding novel na+/h+ antiporters |
| 135 | 20040226 | 87 | US 20040037846 A1 | Chlamydia pmp proteins, gene sequences and uses thereof |
| 136 | 20040219 | 234 | US 20040034196 A1 | 98 human secreted proteins |

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| 137 | 20040219 | 375 | US 20040033582 A1 | Human single nucleotide polymorphisms |
| 138 | 20040219 | 19 | US 20040033564 A1 | Method for increasing solubility of target protein using RNA-binding protein as fusion partner |
| 139 | 20040219 | 311 | US 20040033506 A1 | Polynucleotides encoding novel human mitochondrial and microsomal glycerol-3-phosphate acyl-transferases and variants thereof |
| 140 | 20040219 | 86 | US 20040033230 A1 | Compositions and methods for the therapy and diagnosis of breast cancer |
| 141 | 20040212 | 178 | US 20040030098 A1 | Polynucleotides encoding novel two splice variants of a human cell surface protein with immunologobulin folds, BGS5G and BGS5I |
| 142 | 20040212 | 37 | US 20040029781 A1 | Affinity peptides and method for purification of recombinant proteins |
| 143 | 20040205 | 73 | US 20040025196 A1 | POSH nucleic acids, polypeptides and related methods |
| 144 | 20040205 | 111 | US 20040024192 A1 | 19 human secreted proteins |
| 145 | 20040205 | 140 | US 20040024183 A1 | Transporters and ion channels |
| 146 | 20040205 | 73 | US 20040023207 A1 | Assays for drug discovery based on microcompetition with a foreign polynucleotide |
| 147 | 20040205 | 72 | US 20040023206 A1 | Methods for chronic disease diagnosis based on microcompetition with a foreign polynucleotide |

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| 148 | 20040205 | 63 | US 20040022764 A1 | Inhibition of microcompetition with a foreign polynucleotide as treatment of chronic disease |
| 149 | 20040129 | 290 | US 20040018976 A1 | Polynucleotide encoding novel human G-protein coupled receptors, and splice variants thereof |
| 150 | 20040129 | 413 | US 20040018969 A1 | Nucleic acids, proteins, and antibodies |
| 151 | 20040129 | 155 | US 20040018591 A1 | Methods and compositions for protein expression and purification |
| 152 | 20040129 | 40 | US 20040018534 A1 | Fused DNA sequence, fused protein expressed from said fused DNA sequence and method for expressing said fused protein |
| 153 | 20040129 | 252 | US 20040018204 A1 | Compositions and methods for WT1 specific immunotherapy |
| 154 | 20040122 | 119 | US 20040014945 A1 | Transporters and ion channels |
| 155 | 20040122 | 216 | US 20040014093 A1 | Polynucleotide encoding a novel cysteine protease of the calpain superfamily, Protease-42 |
| 156 | 20040122 | 181 | US 20040014087 A1 | Molecules for diagnostics and therapeutics |
| 157 | 20040108 | 241 | US 20040005700 A1 | Poroplasts |
| 158 | 20040108 | 50 | US 20040005654 A1 | Method of producing polyvalent antigens |
| 159 | 20040101 | 40 | US 20040002065 A1 | PROTEIN/SOLUBILITY FOLDING ASSESSED BY STRUCTURAL COMPLEMENTATION |

| | Issue Date | Pages | Document ID | Title |
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| 160 | 20040101 | 50 | US 20040001848 A1 | Method of producing disease-specific antigens |
| 161 | 20031225 | 82 | US 20030236209 A1 | Compositions and methods for the therapy and diagnosis of lung cancer |
| 162 | 20031225 | 33 | US 20030235901 A1 | Bacterial mycothiol S-conjugate amidase family |
| 163 | 20031225 | 229 | US 20030235557 A1 | Compositions and methods for WT1 specific immunotherapy |
| 164 | 20031218 | 144 | US 20030232359 A1 | Polynucleotide encoding a novel human G-protein coupled receptor, HGPRBMY40_2 |
| 165 | 20031218 | 240 | US 20030232335 A1 | Minicell-based screening for compounds and proteins that modulate the activity of signalling proteins |
| 166 | 20031218 | 222 | US 20030232056 A1 | Compositions and methods for the therapy and diagnosis of ovarian cancer |
| 167 | 20031218 | 25 | US 20030232022 A1 | P. gingivalis antigenic composition |
| 168 | 20031211 | 54 | US 20030228323 A1 | Novel group B streptococcus antigens |
| 169 | 20031204 | 418 | US 20030224486 A1 | Polynucleotides and polypeptides associated with the NF-kB pathway |
| 170 | 20031204 | 49 | US 20030224476 A1 | Method of producing transglutaminase reactive compound |
| 171 | 20031204 | 170 | US 20030224458 A1 | Novel human G-protein coupled receptor, HGPRBMY23, expressed highly in kidney |
| 172 | 20031204 | 351 | US 20030224450 A1 | Polynucleotide encoding a novel TRP channel family member, LTRPC3, and splice variants thereof |

| | Issue Date | Pages | Document ID | Title |
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| 173 | 20031204 | 240 | US 20030224444 A1 | Antibodies to native conformations of membrane proteins |
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